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RATS' PREFERENCES FOR AN ANALGESIC COMPARED TO WATER: AN ALTERNATIVE TO "KILLING THE RAT SO IT DOES NOT SUFFER"[1]

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Summary.—A common policy in research institutions is to kill rats when they display chronic disabilities or recurrent injuries. These guidelines appear to be derived from an oxymoron that "it's better for a rat to be killed so it does not suffer pain" and from untested assumptions that rats cannot control "pain." In a two-bottle paradigm, 10 rats with a history of brain damage following status epilepticus from a single systemic injection of lithium and pilocarpine were given options to consume freely either tap water or 1 mg/cc of acetaminophen in tap water. During periods of fresh lesions due to persistent gnawing or acute injuries associated with tonic-clonic convulsions, the rats consumed 3 to 10 times the fluid from the bottles containing acetaminophen (equivalent to 5 to 10 extra-strength Tylenol tablets per day for a 70-kg person) relative to periods when no lesions or old lesions were present. These results suggest that rats with chronic injuries sufficient to be terminated according to Animal Care guidelines may be capable of reducing the aversive physiological conditions associated with tissue damage by selecting analgesic treatments.

When using rats in research, their optimal care is essential to minimize the effects of organismic distress and to increase the validity of experimental treatments. The specific policies for when a rat should be killed for "humane reasons" are determined by regulating groups such as the Canadian Council for Animal Care. The criteria for termination are derived from highly subjective impressions by veterinarians rather than by the rat itself. Consequently if a rat displays "wheezing," dorsoflexion of the back, a skin lesion, or marked weight loss with piloerection, it is considered "in pain." The underlying philosophy appears to be derived from the premise that "death is better than chronic pain."

Although most professionals would not argue that a human being should be "terminated" because he "appears to be in pain" due to excessive growth of a cancer or significant tissue damage, this argument is routinely applied as a rationale to kill rats involved in research protocols. Frequent discussion with administrative personnel who formulate these policies indicate they are derived from the belief that rats are not capable of discerning internal states and must be "helped" to die so they do not "suffer."

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Pharmacological research has repeatedly shown that rats can differentiate even subtle neurochemical conditions (Appel, White, & Holohean, 1982). The purpose of the present study was to discern whether rats with significant self-inflicted tissue damage due to chronic conditions, such as the brain damage secondary to lithium and pilocarpine-induced epilepsy, could discriminate the consequences of consuming the analgesic acetaminophen. These rats also display spontaneous epileptic seizures during their entire life. Some of the tonic-clonic and picrotoxin-like seizures are associated with body injuries such as removed toenails (Persinger, Bureau, Kostakos, Peredery, & Falter, 1993). If this capacity for discrimination were supported empirically, then the argument that rats must be killed so they do not suffer would have questionable validity.

Demonstrating reliable discrimination does not require the assumption of the same quality of "self-awareness" of pain that occurs with human beings. From an operant perspective, physiological conditions associated with tissue damage should be aversive. If the pharmacological changes associated with consumption of acetaminophen resulted in an attenuation of these aversive interoceptive stimuli, then the response (drinking from a specific bottle) which preceded the removal of the aversive stimuli would be negatively reinforced. Consequently, the response rate should increase. This experimenter predicted that were the above assumptions valid, rats should consume more fluid containing acetaminophen during periods of acute tissue damage. Because the effect was assumed to be very large, only a small sample of rats with the most serious self-inflicted injuries was studied.

Method

Subjects

A total of 10 male Wistar rats, approximately 150 to 300 days of age (450 to 550 gm) were employed as subjects. Males have been employed in our research primarily because they reliably display seizures in response to the experimental dosages of lithium and pilocarpine. They had been obtained from Charles River Suppliers (Quebec). Between 90 and 100 days of age all rats had received 3 mEq/kg of lithium chloride followed either 4 hr. or 24 hr. later by 30 mg/kg of pilocarpine. One hour later (about 20 min. after emergence of *status epilepticus*) the rats had been injected with 30 mg/kg of acepromazine. All drugs were administered subcutaneously.

Procedure

All rats were housed singly in wire cages (43 cm × 24 cm by 17 cm in height). The health and conditions of the rats were monitored by direct observation for between 1 and 3 min. once per day seven days per week. Food (Purina Rat Chow) and water were freely consumed and monitored by the

experimenter and the two animal technicians. Direct observation of the rats' fur (to discern the presence of piloerection), grooming (as inferred by the cleanliness of the fur), and responsivity to stimuli were included in the assessment. These ordinal measures ranged from 0 (normal) to 3 (most severe) and were based upon the guidelines of the facility's veterinarian and the experimenter's personal experience. The rank-order correlations of agreements between observers exceeded *rhos* of .90.

For each block of the experiment, water and acetaminophen were made available in separate 100-cc graduated bottles. The source solution for the analgesia was made by grinding two gel capsules of extra-strength Tylenol (500 mg of acetaminophen per capsule). The powder was added to 1000 cc of tap water (1 mg/cc) and mixed by magnetic vortex for 1 hr. Measurements were daily or once every two days. The fluids in the bottles were changed every second day and placed on the cages when they had reached room temperature. For a given rat the position of the bottle containing acetaminophen or water was always fixed. The nozzles were separated by 3 to 4 cm.

In Block 1, five rats whose epilepsy had been induced about two months previously were given options to drink water or acetaminophen for six consecutive days. During this period they displayed no overt or obvious displays of distress or tissue damage. However, all displayed the occasional spontaneous limbic seizure (rearing and rapid forelimb clonus) during this period. The amount of fluid consumed per day was analyzed by two-way analysis of variance with both levels either repeated (days) or within subjects (acetaminophen vs water consumption).

In Block 2, four rats that exhibited bleeding from mild lacerations of the foot pads or tails, likely induced or exacerbated by complications of seizures, were given the two-bottle option for 17 consecutive days. The presence or absence of an obvious lesion per day was indicated.

The daily lesion score ranged between 0 and 10. The range reflected the amount of damage to the total surface area of the tail and hind footpads as well as the severity of the damage (Bureau & Persinger, 1993). Zero indicated no obvious bleeding or swelling whereas 5 indicated conspicuous swelling of the tail and foot pads or a skin lesion, but no evidence of superficial fresh blood. Scores between 6 and 8 were associated with increased amounts of exposure of fresh tissue exposed and some bleeding. Scores of 9 or above indicated loss of part of the tail or the discharge of blood from the tail during a 24-hr. period sufficient to be smeared over the walls of the metal cage (due to the movement of the tail during ambulation).

Persistent gnawing, specifically of the tail, is a frequent but not invariable behavior displayed by rats with this epileptic history (Bureau & Persinger, 1993). In Block 3 of this experiment one rat that had gnawed severely

the caudal three-quarters of its tail was administered the treatment for 12 days until the necrotic tissue detached. According to the guidelines for the Canadian Council on Animal Care as well as the animal technicians, the rat should have been killed because of the "suffering and pain." Although this single rat was an extreme case and was clearly aesthetically aversive because of the putrescent lesions of the tail, the rat was considered an optimal candidate for the treatment rather than termination simply because he displayed the lesion and "looked bad."

Results

The results of the two-tier repeated-measure analysis of variance (water vs analgesia; days) indicated no statistically significant change in fluid consumption ($F_{5,20}=2.33$, $p>.05$) over the six days. The rats drank significantly ($F_{1,4}=16.64$, $p<.01$; $\eta^2=80\%$) more water ($M=27$ cc, $SD=7$ cc) per day than the acetaminophen solution ($M=14$ cc, $SD=7$ cc). The interaction between type of fluid and time was not significant statistically ($F_{5,20}=1.29$, $p>.05$).

The means and standard deviations for the ratio of the amount of acetaminophen-containing fluid consumed on days when lesions were apparent on the body compared to days when there were no lesions for the four rats in Block 2 are shown in Table 1. Each rat displayed lesions (scores between 0 and 5) for various durations. The singular day of bleeding for Rat 8729 was likely to have been related to removal of all of the toenails of the left rear paw during a "running" seizure, similar to those precipitated by picrotoxin.

TABLE 1
Daily Ratios of Consumption of Water Containing Acetaminophen to Only Water For Days On Which Lesions Were Present or Not Present For Four Rats With Histories of Seizures

Rat	No Lesion			Lesion			η^2
	Days	*M*	*SD*	Days	*M*	*SD*	
8913	11	.8	.3	7	3.1	1.7	52%
8729	17	1.0	.5	1	3.2	.0	
8763	13	.7	.6	5	4.7	5.1	35%
8923	9	.7	.4	9	4.3	2.6	51%

The ratio of acetaminophen fluid consumed relative to water was significantly ($p<.01$) greater (all dfs = 1,16) for all of the rats (Fs = 18.93, 19.22, 8.80, and 17.32, respectively). Heterogeneity of variance between the two conditions was also greater (all Bartlett Box values >15.00) for all rats that displayed more than one day of bleeding. The statistically significant ($p<.01$) point-biserial correlations for the ratio of acetaminophen to water consumed on nonlesion and lesion days for the four rats were .73, .74, .60, and

.72, respectively (all $p < .05$). The correlation (r) between the amount of acetaminophen consumed and the rank of severity of injury, even with this restricted range of scores was .60, .78, and .74 (all $ps < .05$) for Rats 8913, 8729, and 8923, respectively.

The consumption of water or acetaminophen for the rat whose tail had been seriously damaged from repeated gnawing is shown in Fig. 1. Visual inspection indicated that during the period of the most severe self-inflicted tissue damage, the rat's consumption of 1 mg/cc of acetaminophen increased from less than 5 cc per day to about 40 cc per day. When the lesions began to heal, as indicated by the score, the consumption of acetaminophen fluid decreased and water increased. The correlations between the ranks of tissue

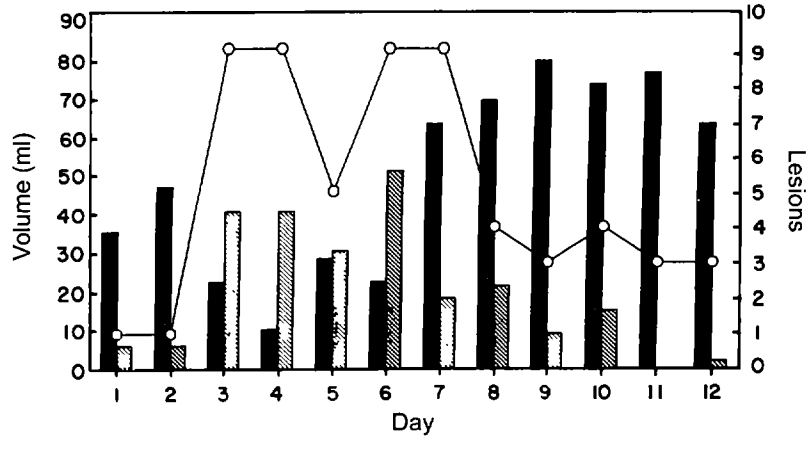

FIG. 1. Volume (in ml) of water (black bars) or Tylenol in water (hatched bars) consumed by a single rat with a self-inflicted lesion (white circle) sufficient to require termination according to guidelines by the Canadian Council for Animal Care

damage and consumption of fluid over the 12 days were .84 ($p < .001$) for the acetaminophen and −.54 ($p < .05$) for water. Removing the shared variance first between the amount of drug consumed and water consumed and the rank and water consumed still indicated a significant partial correlation ($r = .82$, $df = 9$) between the amount of medicated fluid consumed and ranking for severity of injury. The rat did not engage in the gnawing behavior after the treatment and, except for the stump tail, exhibited the typical behavior of other rats with histories of seizures. He died within the age range expected for old rats with seizures.

DISCUSSION

The results of this study are not consistent with the validity of beliefs that laboratory rats who exhibit chronic disabilities, such as swollen foot-

pads or occasional lacerations, should be terminated because the animals can only "experience unavoidable pain." Instead, the results indicated that rats with histories of brain damage due to *status epilepticus* induced by a single systemic injection of lithium and pilocarpine can discriminate the consequences of consuming fluids from either a bottle containing the analgesic acetaminophen or an identical but different bottle containing tap water.

The assumption of "consciousness" or "awareness" of tissue damage or its physiological correlates, such as reported by human beings, is not required to explain this discriminative capacity by rodents. Pain for human beings is an experience that emerges within the cerebral cortices. From this perspective "pain" in human beings is a psychological experience to specific physical stimuli as pitch is to the frequency of sound waves and loudness is to the amplitude of sound waves. Whether or not rats experience pitch or loudness is not required to explain their responses to sound stimuli. Whether rats "experience pain" or do not "experience pain" is not required to explain their responses to tissue damage.

In the present study the rats' behaviors indicated that a specific amount of tissue damage, as inferred by observation, was aversive because rats consumed more analgesia during these periods. From a traditional perspective any response that results in the removal of an aversive stimulus will be repeated and its strength will be increased (negative reinforcement). In this instance, licking the acetaminophen-containing water markedly increased.

The amount of fluid containing acetaminophen consumed per day was positively correlated with the ranked severity of the tissue damage. During days in which bleeding or damage from self-gnawing were present the rat with the greatest tissue damage consumed as much as 40 cc of fluid containing 1 mg/cc of acetaminophen. For a 500-gm rat that would be equivalent to 10 extra-strength Tylenol capsules per day for a 70-kg person. This is within the upper range of recommended dosages employed by humans for continuous general pain or dental discomfort. The results also indicated that ranks of 3 or below, associated with chronic inflammation or edema of the hind foot pads but not bleeding, were not aversive. However, daily consumption of 5 cc per day for a 500-gm rat would be equivalent to about one extra-strength Tylenol capsule.

Although the results of this study clearly indicate rats can discriminate the internal states correlated with a range of tissue damage, this capacity may be an unusual property of brain-damaged rats that produces self-inflicting lesions. The observed effects might also be associated with some anomalous alterations in gustatory preference or threshold. In this instance the same neuroelectrical activity that produces the occasional spontaneous seizures, the self-gnawing, and the tissue damage might also increase the rats' preference for any fluid with gustatory properties other than water. Such al-

tered palatability is frequently but transiently displayed by rats following lesions in the lateral hypothalamus.

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